# Art. XXIV.—*Protozoa Parasitic in the Large Intestine of Australian Frogs.*

## Part II.

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(With Plates LXX. and LXXI.).

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In continuing my investigations into the Protozoa living in the large intestine of Australian frogs, I have obtained for examination, besides additional specimens of those frogs mentioned in Part I., the following:—*Hyla lesueurii* (several specimens); *Helioporus pictus*, *Pseudophryne bibronii*, *Crinia signifera* and *Crinia froggatti* (two specimens of each); *Pseudophryne semimarmorata* and *Hyperolia marmorata* (one specimen of each).

In these last seven species I have found nothing new with the exception of a new *Opalina* in *C. signifera* and a new *Amoeba* in *Hyla lesueurii*. I have also to describe two more new species of *Opalina* from *Limnodynastes dorsalis*, together with some abnormal forms from a frog of the same species. Most of the frogs contain the usual *Trichomonads*, *Nyctotherus cordiformis*, and one or more species of *Opalina*. The new *Amoeba* from *Hyla lesueurii* also occurred in *H. aurea* and *H. peronii*, as well as in tadpoles from various localities.

### Opalina tenuis, n. sp.

This binucleated form was found in the large intestine of two specimens of the brown froglet, *Crinia signifera*, one coming from Narbethong, near Healesville, the other from Mentone, for both of which my thanks are due to Miss O. B. Davies. B.Sc. I have also found it in another small frog from Narbethong, which I take to be *Hyperolia marmorata*. They were present in

very large numbers, and measured on an average 530$\mu$ long, and 36$\mu$ broad. They present a very attenuated or drawn-out appearance, and exhibit the usual corkscrew-like motion (Plate LXX., Fig. 1). The cilia are very long and of much the same length all over the body (Plate LXX., Fig. 2), but at the anterior end they are much more numerous and thickly arranged. The anterior nucleus is situated very near the front end of the body, while the posterior one lies in about the middle of its length, or slightly in the posterior half (Plate LXX., Fig. 1). In the younger and shorter forms the position of the anterior nucleus varies, the individual represented in Plate LXX., Fig. 2, having it situated further down the body than is usual in the larger forms. This individual measured 215$\mu$ in length. The smallest forms measured 100$\mu$ in length. *O. tenuis* occurred along with *O. intestinalis* in *Hyperolia marmorata*, but in *Crinia signifera* it was the only species of *Opalina* present.

## Opalina dorsalis, n. sp.

The species of *Opalina* previously found in *Limnodynastes dorsalis* are *O. intestinalis* and *O. binucleata*, the latter being a new one that I described in Part I. In a frog of the same species just examined, however, I found neither of these present, but two different ones, both of which seem to be new. They are binucleated forms, and were present in great numbers along with *Nyctotherus cordiformis*. The larger of the two I propose to call *O. dorsalis* (Plate LXX , Fig. 3). It is elongate in form and has a very broad anterior end, is slightly flattened, and swims along on either side, giving an occasional roll over on to the other side as it progresses. This enables us to see a thin edge which indicates that the animal is not circular in cross section, but broadly oval. It is ciliated all over with very long cilia, and the two nuclei are always situated in the anterior half of the animal. The most distinguishing feature of the species is the greater width of the anterior end as compared with the posterior. It tapers gradually towards the hinder end and terminates in a point. The cilia extend right to the posterior end. The average length is between 358$\mu$ and 430$\mu$, and at its widest portion it measures about 72$\mu$. The one drawn in

the figure shows the characteristic corkscrew-like folds that the animal presents when moving along. It also shows the slight turn of the "head" end that is noticed during locomotion.

The species at first sight resembles *O. intestinalis* slightly in outline and position of the nuclei, but it differs from it in that the anterior end is broader in proportion to the posterior, which tapers more and ends more sharply than in *O. intestinalis*, and in that the body is slightly flattened and so would not be circular in cross section.

## Opalina acuta, n. sp.

This other new species from *Limnodynastes dorsalis* is much smaller than *O. dorsalis*, and tapers very much towards the posterior end, forming a long tail-like process (Plate LXX., Fig. 4). The average length is 257 $\mu$, and the breadth at its widest portion of about 38$\mu$. The anterior nucleus is situated about 57$\mu$ from the front end, and the posterior about 10$\mu$ further on, and generally slightly nearer the ventral surface. The cilia are well developed on the body portion, but gradually diminish in size and number on the posterior process, until for a length varying from 25$\mu$ to 50$\mu$ from the tip of the process there appear to be none. As the animal moves along, the anterior end is slightly raised, and the dorsal surface appears flattened or straight, while the ventral surface is curved. The characteristic feature that distinguishes this species from others is the very long, thin posterior portion.

## Abnormal forms.

In a specimen of *Limnodynastes dorsalis* coming from Heathcote, Vic., I found numerous forms of *Opalinae* varying in shape and also in the number of nuclei present. Their outlines as drawn with the camera lucida are represented in Plate LXX., Fig. 5. They measured from 120$\mu$ to over 240$\mu$ in length, and I think must be regarded as abnormal forms, in which the nuclei have divided irregularly without the body dividing. They possess nuclei from two to eight in number, which do not seem to be arranged in any definite order. Some of them may repre-

sent abnormal individuals of *O. binucleata*, as for instance (a), (b), (c), (d) and perhaps (f); but others seem to be intermediate forms which are not sufficiently defined to identify.

## Entamoeba morula, n. sp.

This new species is found in great numbers along with *Nyctotherus*, *Opalinae*, *Trichomonads* and *Euglenoids*, in the large intestine of tadpoles of *Limnodynastes tasmaniensis* and others. I have not been able to ascertain in all cases the exact species of tadpole, but from the several localities from which they were obtained, as well as from their appearances, I should judge them to be, in addition to *L. tasmaniensis*, probably *L. dorsalis*, *Hyla aurea* and others. I have also found the animal on three different occasions, but in far smaller numbers, in the large intestine of frogs belonging to the species *H. aurea*, from Alexandra, Victoria; *H. lesueurii*, from Gippsland, Victoria; and *H. peronii*, from New South Wales. The following is a description of the amoeba as it appears directly it is taken from the large intestine and examined under the microscope. The amoeboid stage is roughly cylindrical and is distinguishable into a large elongated oval anterior portion, and a small posterior portion which is continually sending out rounded pseudopodia which are mulberry-like in appearance (Plate LXXI., Fig. 1). It is in these posterior pseudopodia, as well as in its habitat, that *E. morula* differs mainly from other allied forms. This new species is exceedingly active, and creeps along at a remarkable rate, only rarely remaining stationary. The average length is about 125$\mu$, and the average breadth about one-third or one-quarter of its length, but as the shape changes quickly it is difficult to make accurate measurements. I measured one individual that was killed in osmic vapour and mounted in glycerine, that reached 166$\mu$. The protoplasm is only rarely (see below) clearly differentiated into ectoplasm and endoplasm, the contents being as a rule scattered evenly throughout the body, excepting in the small posterior region, which is generally free from the larger granules. A very finely granulated appearance is generally seen in this portion, but at times it appears quite homogeneous and jelly-like. There is a single large nucleus

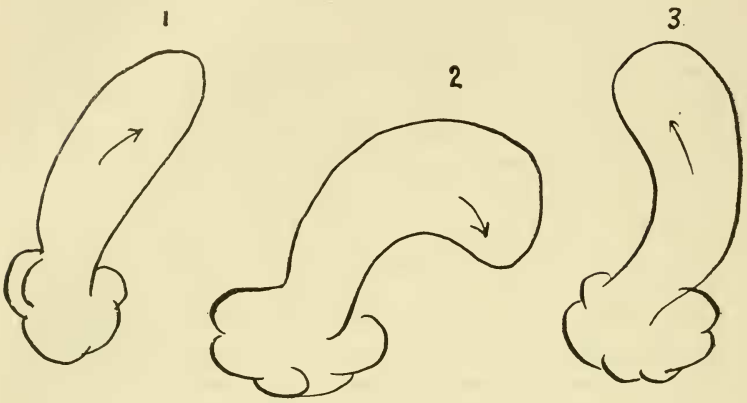
which remains at the front end of the body during movement, but it is only faintly visible in the living animal (Plate LXXI., Fig 1). When stained in picro-carmine it shows up very clearly and is seen to be made up of a central dense granulated portion, a clear homogeneous peripheral portion, and a nuclear membrane closely investing the latter. It measures roughly $9\mu$ in diameter. A contractile vacuole is sometimes visible (Plate LXXI., Fig. 6). In some instances individuals obtained from the tadpoles showed a very bright appearance owing to numerous green and brown bodies present in the protoplasmic stream. These inclusions appeared to be algal spores, diatoms, desmids or their cases, sand grains and other small pieces of débris. Although these were present in large numbers, the amoebae seemed to carry them along with great ease. Others again were found containing colourless oily contents only. Those found living in the frog never contained any green bodies, the protoplasm consisting mainly of ordinary small food vacuoles.

The protoplasm streams very rapidly and can be traced along the middle of the body to the anterior end and then back along the sides, but not extending into the posterior region.

When the amoeba is moving along, it always advances in a definite direction with the larger anterior end foremost. It assumes various shapes but retains as a whole a more or less elongated form (Plate LXXI., Figs. 1, 2, 3, 4 and 5). In creeping forwards a very slight bulging occurs at the front end, which appears to be made of ectoplasm only, being quite free from the larger granules. The protoplasm at once streams into this portion, and the whole individual is thus drawn forward. The contents stream so rapidly into the advancing portion that generally there is no actual pseudopodium (ectoplasmic) visible. The very front end of the amoeba appears to roll over gently and smoothly in a forward direction, and this seems to extend the animal slightly; with this action and with the protoplasm streaming ahead, the individual advances. Only occasionally have I observed the clear portion of the ectoplasm remaining at the front end where the protoplasm had not yet flowed into the projecting pseudopodium, and this is the only time at which ectosarc and endosarc are clearly separate. The direction of movement is changed by the bulging or rolling motion taking

place to one side instead of at the front end, and the protoplasm streaming towards that direction (text figure 1).

Fig. 1.




While the amoeba is thus advancing, a remarkable movement is going on in the posterior portion. It remains clear and jelly-like, but puts out continuously numerous rounded pseudopodia which merge into one another as soon as they are projected, and are renewed incessantly, giving rather a mulberry-like appearance (Plate LXXI., Figs. 1 and 2).

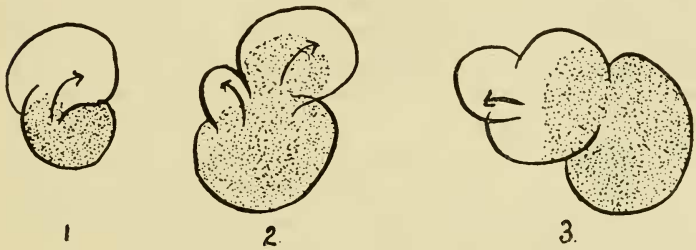
At times each individual pseudopodium is not visible, the whole forming a clear, oily-looking mass (Plate LXXI., Figs. 3 and 4).

Another peculiarity of this posterior region is that it appears to be able to act as a kind of sucker, anchoring the individual down to some support from which it can extend in any direction. I have also observed egestion take place at this region. The waste material is gradually discharged into it, and by the action of the pseudopodia is ultimately egested.

When the animal has been moving about for some time the differentiation into anterior and posterior portions disappears, and the amoeba becomes oval or spherical. This state of rest is generally assumed by them soon after they are removed from the intestine and put into saline solution, and one in this stage killed in osmic vapour and stained in picro-carmine is represented in Plate LXXI., Fig. 6. It measures about 60$\mu$ in diameter. The

contractile vacuole is clearly visible. Occasionally I have observed an individual which has lost its usual form, but is still projecting pseudopodia. There is no indication of the differentiation into anterior and posterior parts left, the body being roughly rounded. The pseudopodia are round and large, and are thrust out rapidly, but there is no locomotion. The contents are propelled suddenly into these pseudopodia as they arise (text figure 2).

Fig. 2.




This may be an abnormality. Plate LXXI., Fig. 7, represents an individual killed in osmic vapour, showing two nuclei at the front end, one of which is slightly larger than the other. It is the only binucleated example I have come across.

In naming the animal I have placed it under the genus *Entamoeba*, which consists of species which are parasitic in the intestines of animals, in contrast to the genus *Amoeba*, which is considered to contain fresh water and marine forms only. Doflein (2) uses these two genera, and although Walker in 1908 (6) regarded the division as unwarranted, I prefer to follow Doflein's classification. In Walker's paper he says:—"Casagrandi and Barbagallo in 1897 proposed the generic name of *Entamoeba* for amoebae parasitic in the intestinal tracts of animals. This new genus *Entamoeba* has been accepted by Schaudinn (1903)." He goes on to say that it seems probable that the parasitic amoebae exhibit all degrees of parasitism from species restricted to one host, to species that can live either in various hosts or free, and because of this he thinks the genus *Entamoeba* does not appear to have any reason for existence.

Comparing *E. morula* with Grassi's species, *E. ranarum*

which is parasitic in *Rana esculenta*, we see no resemblances at all, and *E. morula* is fully four times the size.

It resembles, however, the freshwater amoebae, such as *A. limax*, *A. villosa* and *A. pilosa* in being roughly distinguishable into anterior and posterior portions, and in having specially modified pseudopodia at the posterior end. These pseudopodia vary in the different species, and help to form the specific differences. These species are figured by Cash (1), and in his description of *A. limax* he notes that Penard points out a "peculiar habit of changing the direction of its march by a movement of the anterior portion of the body 'en masse' to the right or left, whilst the posterior portion remains stationary." This seems to show a resemblance to the peculiar way in which, as I have already noted, *E. morula* can be anchored down by the posterior portion.

The figures on Plates I. and II. of *A. villosa* given by Leidy (4) resemble very much the appearance of *E. morula* so far as the anterior end of the animal is concerned, during locomotion, but the posterior end is, of course, entirely different.

## Additional note on Entamoeba morula.

Having completed this short paper, and still having a few more *Limnodynastes dorsalis* tadpoles, I examined again the intestinal contents of four or five individuals and found *Entamoeba morula* still present in large numbers, but a peculiar point was noticed for the first time—namely, the presence of a stiff process or flagellum-like organ situated at the anterior end (Plate LXXI., Fig 8). It seemed to project from the body at almost any angle, and was quite stiff and motionless. Only by the protoplasm of the front of the body moving or rolling over was the position of the flagellum altered. There generally seems to be a slight extension of the protoplasm forming a tiny conical projection which bears the flagellum. This is always situated close to the nucleus and, in some cases, seemed almost to be connected with it. The specimens which showed the flagellum best were those that were advancing very slowly. With close observation on those progressing rapidly, at first one would say there was no sign of a flagellum being present, but in

three or four instances, after close watching, the animal moved into a certain position and the organ became visible. It may be that it is always present, but invisible owing to the fact that during more rapid forward movement of the protoplasm it becomes trailed underneath or above the anterior end of the body. The fineness of the process and the granular nature of the protoplasm prevented me from seeing it, excepting when it was lying or directed away from the body. I have been successful in killing and mounting only a few of these showing the flagellum, and on examining again the specimens mounted a few weeks ago, on which, along with the living animal, the first description was made, I can see no trace whatever of the organ. It may therefore be present at certain times only. In killing and mounting, however, as it is so very fine, it might easily be lost.

The individuals just examined possessing this flagellum had very few posterior pseudopodia, and sometimes none at all. The streaming was generally very rapid, and in some specimens there seemed to be a small portion at the extreme anterior end into which the protoplasm did not flow (Plate LXXI, Fig. 9). In this portion the nucleus was situated.

## LITERATURE.

Same as in Part I., and in addition.

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3. Lankester, E. Ray.—A Treatise on Zoology, Part I. The Lobosa, by Prof. S. J. Hickson, M.A., F.R.S.

4. Leidy, J.—Freshwater Rhizopods of North America, In.; U.S. Geol. Survey of the Territories. Vol. 12, 1879.

5. Raff, J. W.—"Protozoa Parasitic in the Large Intestine of Australian Frogs." Part I. Proc. Roy. Soc. Victoria, vol. xxiii., N.S. Pt. II., 1911.

6. Walker, E. L.—"The Parasitic Amoebae of the Intestinal Tract of Man and Other Animals." Jour. Med. Research, vol. 17 (N.S., vol. 12), 1907.

## EXPLANATION OF PLATES.

### Plate LXX.

All figures drawn with camera lucida.

Fig. 1. Three specimens of *Opalina tenuis*, n.sp., showing general outline and position of nuclei.

Fig. 2. Young individual of same, showing cilia. (High power.)

Fig. 3. Drawing of *Opalina dorsalis*, n.sp., showing ciliation, outline and position of nuclei.

Fig. 4. Same of *Opalina acuta*, n.sp.

Fig. 5. Abnormal forms of *Opalina* from *Limnodynastes dorsalis*.

### Plate LXXI.

A.P. Anterior portion.
C.V. Contractile vacuole.
N. Nucleus.
P.P. Posterior portion.
V.I. Various inclusions.

Fig. 1. Diagram of *Entamoeba morula*, n.sp., drawn from the living animal, showing the characteristic posterior pseudopodia. The nucleus is only faintly visible.

Figs. 2, 3 and 4. Diagrams of same killed in osmic vapour and stained in picro-carmine.

Fig. 5. *E. morula*, after having been left in saline solution for some time, then killed and stained. Posterior pseudopodia disappearing.

Fig. 6. Same, having assumed a spherical shape.

Fig. 7. Individual with two nuclei.

Figs. 8 and 9. *E. morula* showing peculiar anterior flagellum-like organ.